**DHFR-ts in pNIC-Bsa4**

**Oscar Villarreal**

  1 ATGCTCTCCCTGACGCGCATTCTGCGTAAAAAGATCCCGGTTCATGAGCTCGCAGGTAAG  
  61 ATCTCTCGTCCGCCACTCCGTCCATTTTCCGTAGTTGTAGCGAGCGATGAAAAAGGTGGT  
121 ATCGGTGACGGCGGTACCATCCCGTGGGAAATCCCGGAAGACATGCAGTACTTCCGCCGT  
181 GTTACTACCAACCTGCGTGGTAAGAATGTTAAACCGTCTCCATCTAAGCGTAACGCGGTT  
241 GTTATGGGTCGTAAAACCTGGGACAGCCTCCCGCCGAAATTCCGTCCGCTCTCTAACCGC  
301 CTCAATGTTGTTCTGTCTCGTTCCGCTACGAAGGAACAGCTCCTCGCGGGTATCCCAGAC  
361 CCGATCAAGCGCGCAGAAGCGGCGAACGACGTTGTTGCGGTTAACGGTGGTCTCGAGGAT  
421 GCACTGCGTATGCTGGTTTCTAAAGAGCACACCTCTTCTATCGAAACCGTTTTCTGCATC  
481 GGTGGTGGCACCATCTATAAACAGGCCCTGTGTGCCCCGTGTGTAAACGTTCTGCAGGCG  
541 ATCCACCGTACCGTTGTTCGTCCGGCGTCTAACTCTTGCTCTGTTTTCTTCGACATCCCG  
601 GCAGCTGGCACTAAAACGCTCGAAGGTCTGGAACTCGTTCGTGAATCTATCACCGACGAG  
661 CGTGTTTCTACCGGCGCAGGCGGCAAAAAGTACCAGTTCGAAAAACTCGTGCCACGTAAT  
721 TCTGAAGAAGAACAATACCTCAATCTCGTAGGTCGTATCATCGACGAAGGTTGCACCAAG  
781 TGCGACCGCACGGGTGTTGGTACCCGTTCTCTGTTCGGTGCGCAGATGCGTTTCTCTCTC  
841 CGCAATAATCGTCTGCCACTGCTGACCACCAAACGTGTGTTCTGGCGTGGTGTTTGCGAA  
901 GAACTGCTGTGGTTCCTGCGCGGTGAAACCAACGCGAAACTGCTCTCTGACAAGGGTATT  
961 CATATTTGGGACGGTAACGGTTCTCGTGCGTTCCTCGACTCTCGTGGTCTGACCGACTAC  
1021 GACGAGATGGACCTGGGTCCGGTTTACGGTTTTCAGTGGCGTCACTTCGGCGCTGATTAC  
1081 ATCTCTTGCAAAGAAGACTCTGAAGGTAAGGGCGTTGACCAGATCGCGAACATCGTTAAA  
1141 TCTCTGATCGAAAACCCTGATGACCGTCGCATGATCTGCACCGCGTGGAACCCTGCGGCC  
1201 CTGCCGCGCATGGCGCTGCCACCGTGTCACATGATGGCCCAATTCTATGTTTCCAACGGT  
1261 GAACTGTCTTGCATGCTGTACCAGCGTTCTTGCGACATGGGTCTCGGTGTTCCGTTCAAC  
1321 ATCGCCTCTTACGCGCTGCTCACCTTCCTCATGGCCAAAGCGTCTGGTCTCCGCCCTGGT  
1381 GAGCTGGTTCACACCCTGGGCGACGCTCACGTTTATTCTAACCACGTTGAACCGTGCCGT  
1441 AAGCAGCTGAAGCGTGTACCGCGTCCGTTCCCGTTCATTGTTTTCAAGCAAGATAAGGAG  
1501 TTTCTGGAAGATTTCCAGGAGTCTGACATCGAAGTTATCGACTACTCTCCATACCCGGTT  
1561 ATCTCTATGGAAATGGCGGTGTAA

Without Extra Stuff:

ATGCTCTCCCTGACGCGCATTCTGCGTAAAAAGATCCCGGTTCATGAGCTCGCAGGTAAG

ATCTCTCGTCCGCCACTCCGTCCATTTTCCGTAGTTGTAGCGAGCGATGAAAAAGGTGGT

ATCGGTGACGGCGGTACCATCCCGTGGGAAATCCCGGAAGACATGCAGTACTTCCGCCGT

GTTACTACCAACCTGCGTGGTAAGAATGTTAAACCGTCTCCATCTAAGCGTAACGCGGTT

GTTATGGGTCGTAAAACCTGGGACAGCCTCCCGCCGAAATTCCGTCCGCTCTCTAACCGC

CTCAATGTTGTTCTGTCTCGTTCCGCTACGAAGGAACAGCTCCTCGCGGGTATCCCAGAC

CCGATCAAGCGCGCAGAAGCGGCGAACGACGTTGTTGCGGTTAACGGTGGTCTCGAGGAT

GCACTGCGTATGCTGGTTTCTAAAGAGCACACCTCTTCTATCGAAACCGTTTTCTGCATC

GGTGGTGGCACCATCTATAAACAGGCCCTGTGTGCCCCGTGTGTAAACGTTCTGCAGGCG

ATCCACCGTACCGTTGTTCGTCCGGCGTCTAACTCTTGCTCTGTTTTCTTCGACATCCCG

GCAGCTGGCACTAAAACGCTCGAAGGTCTGGAACTCGTTCGTGAATCTATCACCGACGAG

CGTGTTTCTACCGGCGCAGGCGGCAAAAAGTACCAGTTCGAAAAACTCGTGCCACGTAAT

TCTGAAGAAGAACAATACCTCAATCTCGTAGGTCGTATCATCGACGAAGGTTGCACCAAG

TGCGACCGCACGGGTGTTGGTACCCGTTCTCTGTTCGGTGCGCAGATGCGTTTCTCTCTC

CGCAATAATCGTCTGCCACTGCTGACCACCAAACGTGTGTTCTGGCGTGGTGTTTGCGAA

GAACTGCTGTGGTTCCTGCGCGGTGAAACCAACGCGAAACTGCTCTCTGACAAGGGTATT

CATATTTGGGACGGTAACGGTTCTCGTGCGTTCCTCGACTCTCGTGGTCTGACCGACTAC

GACGAGATGGACCTGGGTCCGGTTTACGGTTTTCAGTGGCGTCACTTCGGCGCTGATTAC

ATCTCTTGCAAAGAAGACTCTGAAGGTAAGGGCGTTGACCAGATCGCGAACATCGTTAAA

TCTCTGATCGAAAACCCTGATGACCGTCGCATGATCTGCACCGCGTGGAACCCTGCGGCC

CTGCCGCGCATGGCGCTGCCACCGTGTCACATGATGGCCCAATTCTATGTTTCCAACGGT

GAACTGTCTTGCATGCTGTACCAGCGTTCTTGCGACATGGGTCTCGGTGTTCCGTTCAAC

ATCGCCTCTTACGCGCTGCTCACCTTCCTCATGGCCAAAGCGTCTGGTCTCCGCCCTGGT

GAGCTGGTTCACACCCTGGGCGACGCTCACGTTTATTCTAACCACGTTGAACCGTGCCGT

AAGCAGCTGAAGCGTGTACCGCGTCCGTTCCCGTTCATTGTTTTCAAGCAAGATAAGGAG

TTTCTGGAAGATTTCCAGGAGTCTGACATCGAAGTTATCGACTACTCTCCATACCCGGTT

ATCTCTATGGAAATGGCGGTGTAA

With Stuff Added:

TACTTCCAATCCATGCTCTCCCTGACGCGCATTCTGCGTAAAAAGATCCCGGTTCATGAGCTCGCAGGTAAG

ATCTCTCGTCCGCCACTCCGTCCATTTTCCGTAGTTGTAGCGAGCGATGAAAAAGGTGGT

ATCGGTGACGGCGGTACCATCCCGTGGGAAATCCCGGAAGACATGCAGTACTTCCGCCGT

GTTACTACCAACCTGCGTGGTAAGAATGTTAAACCGTCTCCATCTAAGCGTAACGCGGTT

GTTATGGGTCGTAAAACCTGGGACAGCCTCCCGCCGAAATTCCGTCCGCTCTCTAACCGC

CTCAATGTTGTTCTGTCTCGTTCCGCTACGAAGGAACAGCTCCTCGCGGGTATCCCAGAC

CCGATCAAGCGCGCAGAAGCGGCGAACGACGTTGTTGCGGTTAACGGTGGTCTCGAGGAT

GCACTGCGTATGCTGGTTTCTAAAGAGCACACCTCTTCTATCGAAACCGTTTTCTGCATC

GGTGGTGGCACCATCTATAAACAGGCCCTGTGTGCCCCGTGTGTAAACGTTCTGCAGGCG

ATCCACCGTACCGTTGTTCGTCCGGCGTCTAACTCTTGCTCTGTTTTCTTCGACATCCCG

GCAGCTGGCACTAAAACGCTCGAAGGTCTGGAACTCGTTCGTGAATCTATCACCGACGAG

CGTGTTTCTACCGGCGCAGGCGGCAAAAAGTACCAGTTCGAAAAACTCGTGCCACGTAAT

TCTGAAGAAGAACAATACCTCAATCTCGTAGGTCGTATCATCGACGAAGGTTGCACCAAG

TGCGACCGCACGGGTGTTGGTACCCGTTCTCTGTTCGGTGCGCAGATGCGTTTCTCTCTC

CGCAATAATCGTCTGCCACTGCTGACCACCAAACGTGTGTTCTGGCGTGGTGTTTGCGAA

GAACTGCTGTGGTTCCTGCGCGGTGAAACCAACGCGAAACTGCTCTCTGACAAGGGTATT

CATATTTGGGACGGTAACGGTTCTCGTGCGTTCCTCGACTCTCGTGGTCTGACCGACTAC

GACGAGATGGACCTGGGTCCGGTTTACGGTTTTCAGTGGCGTCACTTCGGCGCTGATTAC

ATCTCTTGCAAAGAAGACTCTGAAGGTAAGGGCGTTGACCAGATCGCGAACATCGTTAAA

TCTCTGATCGAAAACCCTGATGACCGTCGCATGATCTGCACCGCGTGGAACCCTGCGGCC

CTGCCGCGCATGGCGCTGCCACCGTGTCACATGATGGCCCAATTCTATGTTTCCAACGGT

GAACTGTCTTGCATGCTGTACCAGCGTTCTTGCGACATGGGTCTCGGTGTTCCGTTCAAC

ATCGCCTCTTACGCGCTGCTCACCTTCCTCATGGCCAAAGCGTCTGGTCTCCGCCCTGGT

GAGCTGGTTCACACCCTGGGCGACGCTCACGTTTATTCTAACCACGTTGAACCGTGCCGT

AAGCAGCTGAAGCGTGTACCGCGTCCGTTCCCGTTCATTGTTTTCAAGCAAGATAAGGAG

TTTCTGGAAGATTTCCAGGAGTCTGACATCGAAGTTATCGACTACTCTCCATACCCGGTT

ATCTCTATGGAAATGGCGGTGTAACAGTAAAGGTGGATA

Upstream and Downstream Stuff:

Upstream: add TACTTCCAATCCATG to the 5’ end (ATG in-frame with the desired coding sequence). Downstream: add TATCCACCTTTACTG to 5’ end of downstream primer; add termination codon, if necessary.

pNIC-BSA4 Sequence:

TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTT

TAAGAAGGAGATATACATATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACC

TGTACTTCCAATCCATGCTCTCCCTGACGCGCATTCTGCGTAAAAAGATCCCGGTTCATGAGCTCGCAGGTAAG

ATCTCTCGTCCGCCACTCCGTCCATTTTCCGTAGTTGTAGCGAGCGATGAAAAAGGTGGT

ATCGGTGACGGCGGTACCATCCCGTGGGAAATCCCGGAAGACATGCAGTACTTCCGCCGT

GTTACTACCAACCTGCGTGGTAAGAATGTTAAACCGTCTCCATCTAAGCGTAACGCGGTT

GTTATGGGTCGTAAAACCTGGGACAGCCTCCCGCCGAAATTCCGTCCGCTCTCTAACCGC

CTCAATGTTGTTCTGTCTCGTTCCGCTACGAAGGAACAGCTCCTCGCGGGTATCCCAGAC

CCGATCAAGCGCGCAGAAGCGGCGAACGACGTTGTTGCGGTTAACGGTGGTCTCGAGGAT

GCACTGCGTATGCTGGTTTCTAAAGAGCACACCTCTTCTATCGAAACCGTTTTCTGCATC

GGTGGTGGCACCATCTATAAACAGGCCCTGTGTGCCCCGTGTGTAAACGTTCTGCAGGCG

ATCCACCGTACCGTTGTTCGTCCGGCGTCTAACTCTTGCTCTGTTTTCTTCGACATCCCG

GCAGCTGGCACTAAAACGCTCGAAGGTCTGGAACTCGTTCGTGAATCTATCACCGACGAG

CGTGTTTCTACCGGCGCAGGCGGCAAAAAGTACCAGTTCGAAAAACTCGTGCCACGTAAT

TCTGAAGAAGAACAATACCTCAATCTCGTAGGTCGTATCATCGACGAAGGTTGCACCAAG

TGCGACCGCACGGGTGTTGGTACCCGTTCTCTGTTCGGTGCGCAGATGCGTTTCTCTCTC

CGCAATAATCGTCTGCCACTGCTGACCACCAAACGTGTGTTCTGGCGTGGTGTTTGCGAA

GAACTGCTGTGGTTCCTGCGCGGTGAAACCAACGCGAAACTGCTCTCTGACAAGGGTATT

CATATTTGGGACGGTAACGGTTCTCGTGCGTTCCTCGACTCTCGTGGTCTGACCGACTAC

GACGAGATGGACCTGGGTCCGGTTTACGGTTTTCAGTGGCGTCACTTCGGCGCTGATTAC

ATCTCTTGCAAAGAAGACTCTGAAGGTAAGGGCGTTGACCAGATCGCGAACATCGTTAAA

TCTCTGATCGAAAACCCTGATGACCGTCGCATGATCTGCACCGCGTGGAACCCTGCGGCC

CTGCCGCGCATGGCGCTGCCACCGTGTCACATGATGGCCCAATTCTATGTTTCCAACGGT

GAACTGTCTTGCATGCTGTACCAGCGTTCTTGCGACATGGGTCTCGGTGTTCCGTTCAAC

ATCGCCTCTTACGCGCTGCTCACCTTCCTCATGGCCAAAGCGTCTGGTCTCCGCCCTGGT

GAGCTGGTTCACACCCTGGGCGACGCTCACGTTTATTCTAACCACGTTGAACCGTGCCGT

AAGCAGCTGAAGCGTGTACCGCGTCCGTTCCCGTTCATTGTTTTCAAGCAAGATAAGGAG

TTTCTGGAAGATTTCCAGGAGTCTGACATCGAAGTTATCGACTACTCTCCATACCCGGTT

ATCTCTATGGAAATGGCGGTGTAACAGTAAAGGTGGATACGG

ATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCC

GGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCC

CTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATTGGCGAATG

GGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTT

GCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCC

GTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAA

ACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTG

GAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATT

CTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATT

TAACGCGAATTTTAACAAAATATTAACGTTTACAATTTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGA

ACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAATTAATTCTTAGAAAAAC

TCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCC

GTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGC

GATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAG

AAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTT

CAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTG

CGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGG

CGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATG

CTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGT

CGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTA

CCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTG

ATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGG

CCTAGAGCAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGAC

AGTTTTATTGTTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAA

AAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCAC

CGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAG

CAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTA

GCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTC

TTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTG

CACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGC

GCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCA

CGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGA

GCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTA

CGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATA

ACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGT

GAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGC

ATATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATACACTCCGCTATCG

CTACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTC

TGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACC

GTCATCACCGAAACGCGCGAGGCAGCTGCGGTAAAGCTCATCAGCGTGGTCGTGAAGCGATTCACAGATG

TCTGCCTGTTCATCCGCGTCCAGCTCGTTGAGTTTCTCCAGAAGCGTTAATGTCTGGCTTCTGATAAAGC

GGGCCATGTTAAGGGCGGTTTTTTCCTGTTTGGTCACTGATGCCTCCGTGTAAGGGGGATTTCTGTTCAT

GGGGGTAATGATACCGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTGATGATGAACATGCCCGG

TTACTGGAACGTTGTGAGGGTAAACAACTGGCGGTATGGATGCGGCGGGACCAGAGAAAAATCACTCAGG

GTCAATGCCAGCGCTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGCATCCTGCGATGCA

GATCCGGAACATAATGGTGCAGGGCGCTGACTTCCGCGTTTCCAGACTTTACGAAACACGGAAACCGAAG

ACCATTCATGTTGTTGCTCAGGTCGCAGACGTTTTGCAGCAGCAGTCGCTTCACGTTCGCTCGCGTATCG

GTGATTCATTCTGCTAACCAGTAAGGCAACCCCGCCAGCCTAGCCGGGTCCTCAACGACAGGAGCACGAT

CATGCGCACCCGTGGGGCCGCCATGCCGGCGATAATGGCCTGCTTCTCGCCGAAACGTTTGGTGGCGGGA

CCAGTGACGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAATACCGCAAGCGACAGGCCGATCATCGTCG

CGCTCCAGCGAAAGCGGTCCTCGCCGAAAATGACCCAGAGCGCTGCCGGCACCTGTCCTACGAGTTGCAT

GATAAAGAAGACAGTCATAAGTGCGGCGACGATAGTCATGCCCCGCGCCCACCGGAAGGAGCTGACTGGG

TTGAAGGCTCTCAAGGGCATCGGTCGAGATCCCGGTGCCTAATGAGTGAGCTAACTTACATTAATTGCGT

TGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGC

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CCGAGATATCCGCACCAACGCGCAGCCCGGACTCGGTAATGGCGCGCATTGCGCCCAGCGCCATCTGATC

GTTGGCAACCAGCATCGCAGTGGGAACGATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGAC

ATGGCACTCCAGTCGCCTTCCCGTTCCGCTATCGGCTGAATTTGATTGCGAGTGAGATATTTATGCCAGC

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TGGTCAGAGACATCAAGAAATAACGCCGGAACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGT

CATCCAGCGGATAGTTAATGATCAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACA

GGCTTCGACGCCGCTTCGTTCTACCATCGACACCACCACGCTGGCACCCAGTTGATCGGCGCGAGATTTA

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GTTTGCCCGCCAGTTGTTGTGCCACGCGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTT

TTCCCGCGTTTTCGCAGAAACGTGGCTGGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCG

GCATACTCTGCGACATCGTATAACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGGC

GCTATCATGCCATACCGCGAAAGGTTTTGCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTAT

GCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCAAGGAATGG

TGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAA

GCGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAA

CCGCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCGAGATCTCGATCCCGCG

AAAT

**Forward Primer:**

TACTTCCAATCCATGCTCTCCCTGACGCGCATTCT

**Reverse Primer:**

TATCCACCTTTACTGTTACACCGCCATTTCCATAGAGAT

Forward Primer:

5’ - TACTTCCAATCCATGCTCTCCCTGACGCGCATT-3’ \_33\_\_ bp

GC Content \_\_51.5\_%

0 mM Mg2+ Tm \_\_66.5\_ oC 1.5 mM Mg2+ Tm \_73.6\_ oC 2 mM Mg2+ Tm \_74.0\_\_ oC

4 mM Mg2+ Tm \_74.7\_\_ oC 6 mM Mg2+ Tm \_75.1\_\_ oC

Reverse Primer:

5’-CTCTATGGAAATGGCGGTGTAACAGTAAAGGTGGATA-3’

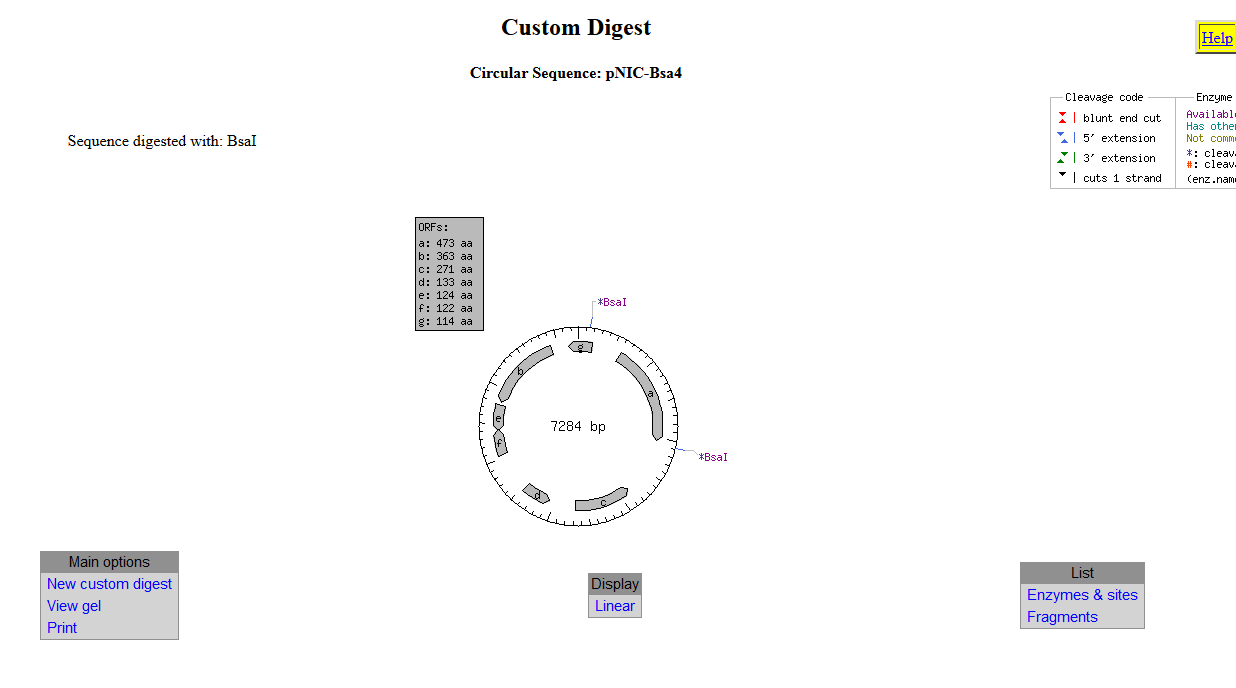
**Reverse complement it:**

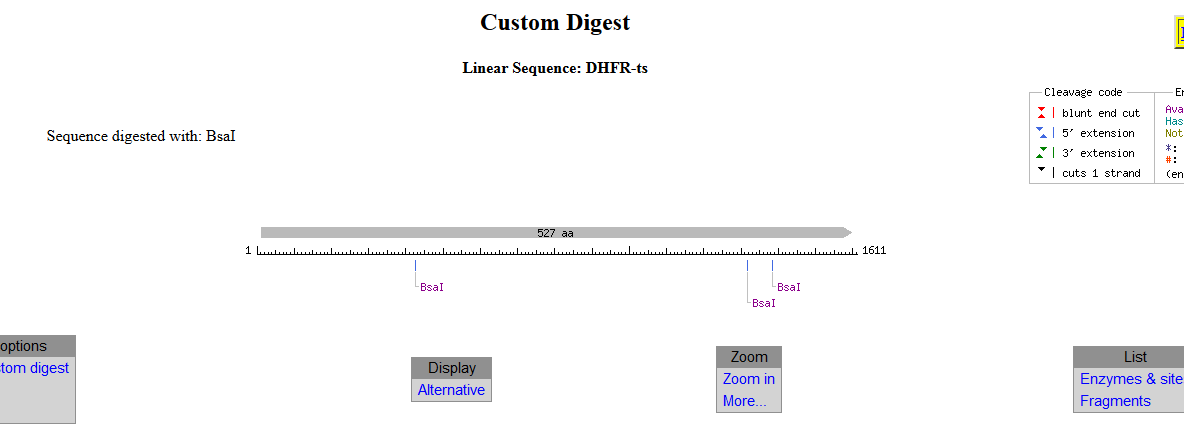
5’-TATCCACCTTTACTGTTACACCGCCATTTCCATAGAG-3’ \_\_37\_ bp

GC Content \_43.2\_\_%

0 mM Mg2+ Tm \_62.8\_\_ oC 1.5 mM Mg2+ Tm \_70.5\_\_ oC 2 mM Mg2+ Tm \_71.6\_ oC

4 mM Mg2+ Tm \_72.4\_\_ oC 6 mM Mg2+ Tm \_72.8\_\_ oC





**Complete Plasmid:**

TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTT

TAAGAAGGAGATATACATATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACC

TGTACTTCCAATCCATGCTCTCCCTGACGCGCATTCTGCGTAAAAAGATCCCGGTTCATGAGCTCGCAGGTAAG

ATCTCTCGTCCGCCACTCCGTCCATTTTCCGTAGTTGTAGCGAGCGATGAAAAAGGTGGT

ATCGGTGACGGCGGTACCATCCCGTGGGAAATCCCGGAAGACATGCAGTACTTCCGCCGT

GTTACTACCAACCTGCGTGGTAAGAATGTTAAACCGTCTCCATCTAAGCGTAACGCGGTT

GTTATGGGTCGTAAAACCTGGGACAGCCTCCCGCCGAAATTCCGTCCGCTCTCTAACCGC

CTCAATGTTGTTCTGTCTCGTTCCGCTACGAAGGAACAGCTCCTCGCGGGTATCCCAGAC

CCGATCAAGCGCGCAGAAGCGGCGAACGACGTTGTTGCGGTTAACGGTGGTCTCGAGGAT

GCACTGCGTATGCTGGTTTCTAAAGAGCACACCTCTTCTATCGAAACCGTTTTCTGCATC

GGTGGTGGCACCATCTATAAACAGGCCCTGTGTGCCCCGTGTGTAAACGTTCTGCAGGCG

ATCCACCGTACCGTTGTTCGTCCGGCGTCTAACTCTTGCTCTGTTTTCTTCGACATCCCG

GCAGCTGGCACTAAAACGCTCGAAGGTCTGGAACTCGTTCGTGAATCTATCACCGACGAG

CGTGTTTCTACCGGCGCAGGCGGCAAAAAGTACCAGTTCGAAAAACTCGTGCCACGTAAT

TCTGAAGAAGAACAATACCTCAATCTCGTAGGTCGTATCATCGACGAAGGTTGCACCAAG

TGCGACCGCACGGGTGTTGGTACCCGTTCTCTGTTCGGTGCGCAGATGCGTTTCTCTCTC

CGCAATAATCGTCTGCCACTGCTGACCACCAAACGTGTGTTCTGGCGTGGTGTTTGCGAA

GAACTGCTGTGGTTCCTGCGCGGTGAAACCAACGCGAAACTGCTCTCTGACAAGGGTATT

CATATTTGGGACGGTAACGGTTCTCGTGCGTTCCTCGACTCTCGTGGTCTGACCGACTAC

GACGAGATGGACCTGGGTCCGGTTTACGGTTTTCAGTGGCGTCACTTCGGCGCTGATTAC

ATCTCTTGCAAAGAAGACTCTGAAGGTAAGGGCGTTGACCAGATCGCGAACATCGTTAAA

TCTCTGATCGAAAACCCTGATGACCGTCGCATGATCTGCACCGCGTGGAACCCTGCGGCC

CTGCCGCGCATGGCGCTGCCACCGTGTCACATGATGGCCCAATTCTATGTTTCCAACGGT

GAACTGTCTTGCATGCTGTACCAGCGTTCTTGCGACATGGGTCTCGGTGTTCCGTTCAAC

ATCGCCTCTTACGCGCTGCTCACCTTCCTCATGGCCAAAGCGTCTGGTCTCCGCCCTGGT

GAGCTGGTTCACACCCTGGGCGACGCTCACGTTTATTCTAACCACGTTGAACCGTGCCGT

AAGCAGCTGAAGCGTGTACCGCGTCCGTTCCCGTTCATTGTTTTCAAGCAAGATAAGGAG

TTTCTGGAAGATTTCCAGGAGTCTGACATCGAAGTTATCGACTACTCTCCATACCCGGTT

ATCTCTATGGAAATGGCGGTGTAACAGTAAAGGTGGATACGG

ATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCC

GGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCC

CTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATTGGCGAATG

GGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTT

GCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCC

GTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAA

ACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTG

GAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATT

CTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATT

TAACGCGAATTTTAACAAAATATTAACGTTTACAATTTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGA

ACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAATTAATTCTTAGAAAAAC

TCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCC

GTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGC

GATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAG

AAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTT

CAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTG

CGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGG

CGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATG

CTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGT

CGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTA

CCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTG

ATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGG

CCTAGAGCAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGAC

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